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SUJET DE LA THÈSE :

BIOSYNTHÈSE DE LA MÉTHIONINE ET SA RÉGULATION CHEZ SACCHAROMYCES CEREVISIAE

ARTICLE PRINCIPAL

recouvrant en partie la thèse de Doctorat ès Sciences Naturelles enregistrée au C. N. R. S. sous le Numéro A. O. 4143.

Hélène CHEREST, née IEVDOKIMOFF

Soutenue le 1970, à la Faculté des Sciences d'Orsay devant le jury :

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Genetic and Regulatory Aspects of Methionine Biosynthesis in *Saccharomyces cerevisiae*

H. CHEREST, F. EICHLER, AND H. DE ROBICHON-SZULMAJSTER
Laboratoire d'Enzymologie, Centre National de la Recherche Scientifique, 91-Gif-sur-Yvette, France

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Methionine biosynthesis and regulation of four enzymatic steps involved in this pathway were studied in *Saccharomyces cerevisiae*, in relation to genes concerned with resistance to ethionine (eth_1 and eth_2). Data presented in this paper and others favor a scheme which excludes cystathionine as an obligatory intermediate. Kinetic data are presented for homocysteine synthetase [K_m(*O*-acetyl-L-homoserine) = 7×10^{-3} M; K_i (L-methionine) = 1.9×10^{-3} M]. Enzymes catalyzing steps 3, 4, 5, and 9 were repressible by methionine. Enzyme 4 (homoserine-*O*-transacetylase) and enzyme 9 (homocysteine synthetase) were simultaneously derepressed in strains carrying the mutant allele eth_2^r. Studies on diploid strains confirmed the dominance of the eth_2^s allele over eth_2^r. Regulation of enzyme 3 (homoserine dehydrogenase) and enzyme 5 (adenosine triphosphate sulfurylase) is not modified by the allele eth_2^r. The other gene eth_1 did not appear to participate in regulation of these four steps. Gene enzyme relationship was determined for three of the four steps studied (steps 3, 4, and 9). The structural genes concerned with the steps which are under the control of eth_2 (met_8:enzyme 9 and met_a: enzyme 4) segregate independently, and are unlinked to eth_2. These results are compatible with the idea that the gene eth_2 is responsible for the synthesis of a pleiotropic methionine repressor and suggest the existence of at least two different methionine repressors in *S. cerevisiae*. Implications of these findings in general regulatory mechanisms have been discussed.

Methionine biosynthesis in *Saccharomyces cerevisiae* can be divided in three parts: (i) synthesis of the four-carbon skeleton; (ii) sulfur assimilation; (iii) synthesis and attachment of the methyl groups. Regulation by end-product inhibition and repression occurs at different steps of this pathway. This paper is concerned only with biochemical and regulatory aspects of parts (i) and (ii).

The four-carbon skeleton of methionine and threonine originates from aspartate. Homoserine dehydrogenase [L-homoserine:nicotinamide adenine dinucleotide (NAD) oxidoreductase; EC 1.1.1.3], the enzyme catalyzing the last common step leading to these two end products, is repressed by methionine (65% repression in the wild-type strains); both methionine and threonine participate in the feedback inhibition of this enzyme (12). Aspartate kinase (L-aspartate 4-phosphotransferase; EC 2.7.2.4), catalyzing the first step in threonine-methionine biosynthesis, is repressed by threonine; a mutation altering this repression does not affect the repressibility of homoserine dehydrogenase (27).

From homoserine, the biosynthesis of methionine starts with an acetylation reaction leading to *O*-acetyl-homoserine which is apparently the active substrate for the entry of sulfur into the pathway. The enzyme that catalyzes homoserine *O*-acetylation (homoserine-*O*-transacetylase) is under the control of methionine for its synthesis and of *S*-adenosyl-methionine for its activity (25).

The sulfur assimilation originates with sulfate activation (22). In yeast, this reaction is subject to feedback inhibition by sulfide. In addition, adenosine 5′-phosphosulfate (APS) and 3-′phospho-adenosine-5′-phosphosulfate (PAPS), which are the intermediary compounds leading to sulfite, are also inhibitors. The synthesis of the first enzyme, adenosine triphosphate sulfurylase (ATP:sulfate adenylyltransferase; EC 2.7.7.4), is repressed by methionine and derepressed by cysteine (35). On the other hand, the enzyme sulfite reductase [hydrogen sulfide:nicotinamide adenine dinucleotide phosphate (NADP) oxidoreductase; EC 1.8.1.2] is repressed in cysteine-grown cells (35). Consequently, sulfide synthesis appears to be under double, and somehow complementary, control of the two sulfur-containing amino acids, methionine and cysteine.

Direct sulfide utilization occurred by condensation of homoserine and H_2S in *Neurospora* (37)

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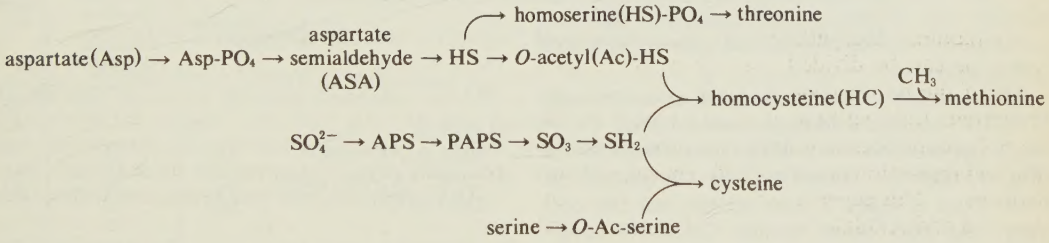
and yeast (38). However, homocysteine is synthesized much more efficiently when acetyl-homoserine, instead of homoserine, is used as a substrate (38). This reaction has also been demonstrated in spinach (10).

Consideration of the role of cystathionine synthesis and cleavage in bacteria (3, 29) had led to the postulation of this compound as an obligatory intermediate in *Neurospora*. In addition to the existence of enzymes able to condense acetyl-homoserine and cysteine into cystathionine and to cleave the latter compound, the hypothesis was based upon nutritional experiments and upon the fact that mutants lacking β-cystathionase are methionine auxotrophs (20).

In yeast, cystathionine synthesis from cysteine and *O*-acetyl-homoserine was not yet clearly demonstrated, and the cystathionine γ-cleavage enzyme (leading to homocysteine) is present to a much lower extent than in *Neurospora* (3).

Moreover, the facts that homocysteine synthetase activity is specifically inhibited by methionine (37, 38) and that the synthesis of this enzyme is repressed by methionine are in the favor of direct synthesis of homocysteine. In addition, this paper shows that a mutant of *S. cerevisiae* missing homocysteine synthetase is unable to synthesize methionine. These considerations led us to favor the following scheme of methionine biosynthesis in *S. cerevisiae*. (See legend of Fig. 3 for abbreviations.)



Regulation studies on methionine-controlled biosynthetic enzymes were greatly facilitated by selection of ethionine-resistant mutants. Three mutations have been studied thus far. One is concerned with amino acid uptake, and confers resistance toward other amino acid analogues, such as para-fluoro-phenylalanine (33). The two other genes, eth_1 and eth_2, govern resistance to ethionine specifically. For one of them, the mutant allele eth_1^r is dominant; for the other, the mutant allele, eth_2^r, is recessive. The eth_1^r phenotype is recognizable only by the fact that it enhances the ethionine resistance conferred by eth_2^r (2, 24).

It has been found recently that eth_2^r is concerned with regulation of the first step in the four-carbon skeleton of methionine homoserine *O*-transacetylase (23, 25, and this paper). The eth_1^r does not influence regulation of this step. Its role is still under study. In addition, we found that eth_2^r is also concerned with regulation of homocysteine synthetase.

MATERIALS AND METHODS

Strains. The haploid strains of *S. cerevisiae* used for this investigation are as follows: 4094-B:α, ade_2, ura_1 (from Sherman's collection); D6:α, met_a, *ura* (from Grenson's collection); EY9:α, met_3; and X 1266-1C:*a*, his_4, leu_2, ura_1, ade_2, met_{10} (from R. K. Mortimer's collection); CH 82-7A:α, ura_1; CC30-1D:α, ade_2, ura_1, CH82-7D:α, ade_2, ura_1; CH82-9C:*a*, ade_2, ura_1; and CH92-8D:*a*, met_a, ade_2, *ura* (from our own collection). [met_a designates the structural gene for homoserine-*O*-transacetylase (25)].

The diploid strains are the following: CC 17:+/ade_2; ura_1/ura_1; eth_1^s/eth_1^r; eth_2^r/eth_2^r. CC 18:ade_2/ade_2; ura_1/ura_1; eth_1^s/eth_1^r; eth_2^s/eth_2^r. CC 53:+/ade_2; ura_1/ura_1; eth_1^s/eth_1^s; eth_2^s/eth_2^r.

Media. The YPGA medium was used for preparation of inocula. It contains, per liter: Difco yeast extract, 10 g; glucose, 20 g; and adenine, 20 mg.

The synthetic medium used was a minimal medium prepared according to Galzy and Slonimski (8) and buffered to *p*H 6.5 according to de Robichon-Szulmajster and Magee (26). This medium was supplemented, when necessary, with adenine, 10 mg/liter; uracil, 10 mg/liter; L-histidine, 100 mg/liter; DL-leucine, 100 mg/liter; DL-methionine, 30 mg/liter. For repression experiments, the concentration of methionine used is given in the text.

Cultures were grown in 1 liter of the appropriate medium, in 2-liter Fernbach flasks inoculated from a 24-hr culture grown on YPGA medium (the inocula were washed twice with sterile water). All cultures were shaken vigorously at 28 C and harvested at optical densities (650 nm) between 1.5 and 2.5.

Extracts. Depending upon the enzyme to be assayed, the cells were washed twice and resuspended in the buffer (*p*H and final concentration) described for each case. Extracts were prepared as previously described (26). Separate extracts were made when necessary.

Enzyme assays. (i) Homoserine dehydrogenase, as described previously (12); (ii) homoserine-*O*-transacetylase, as described by de Robichon-Szulmajster and Cherest (25); (iii) ATP-sulfurylase, as described by de Vito and Dreyfuss (35) either with dialyzed or undialyzed crude extracts; (iv) homocysteine synthetase, incubation carried out according to Wiebers and Garner (38), but at 30 instead of 37 C.

Homocysteine formed was estimated according to Kredich and Tomkins (13).

Protein estimation was carried out according to Lowry et al. (16), with bovine serum albumin as reference.

Genetic techniques. Sporulation of diploids was induced according to McClary et al. (17). Ascospores were isolated according to Johnston and Mortimer (11).

Chemicals. *O*-acetyl-DL-homoserine was synthesized for us by M. Cherest according to Sakami and Toennies (30).

DL-C_4-^{14}C-homoserine was purchased from the C.E.A., France.

Inorganic pyrophosphatase was obtained from Worthington Biochemical Corp., Freehold, N.J.

Aspartic semialdehyde was prepared according to Black and Wright (1) and titered with purified yeast homoserine dehydrogenase (12).

RESULTS

Homoserine O transacetylase. Earlier studies on methionine auxotrophs devoided of homoserine-*O*-transacetylase activity have shown that homoserine-*O*-acetylation is the first step in methionine biosynthesis in *S. cerevisiae* (25). These studies also led to the following conclusions: (i) that activity of this enzyme in crude extracts is subject to feedback inhibition, not by free methionine but by its activated product *S*-adenosyl-methionine; (ii) that the synthesis of homoserine-*O*-transacetylase is repressed by methionine (70% repression by DL-methionine 2×10^{-3} M). In addition, we pointed out the possible role of eth_2^r in this repression. Further investigation on the role of this gene is presented in this paper.

After growth in the presence of DL-methionine (2×10^{-3} M), the wild-type strains 4094-B (eth_1^s, eth_2^s) synthesized very little acetyl-homoserine (Fig. 1, spot 2); moreover, the compound derived from it (spot 3) was absent; on the contrary, the two other strains CH82-7D (eth_1^r, eth_2^r) and CC30-ID (eth_1^s, eth_2^r) synthesized considerable amounts of these two compounds.

A quantitative estimation of homoserine-*O*-transacetylase activity is summarized in Table 1. All strains carrying the wild-type allele (eth_2^s) were repressible by methionine, whereas the strains carrying the mutated recessive allele (eth_2^r) lost this repressibility. The results show unequivocally that the alleles eth_1^r or eth_1^s do not modify this regulation. In either case, *S*-adenosyl-methionine inhibition is maintained. Furthermore, Table 1 shows the absence of homoserine-*O*-transacetylase in mutant strains D6 and CC92–8D (gene met_a).

Homocysteine synthetase. Direct synthesis of homocysteine has been shown to occur in *Neurospora* from homoserine and H_2S by Wiebers and

TABLE 1. *Homoserine-O-transacetylase activity[a] and repression in different strains of S. cerevisiae[b]*

Strains	Genotypes	Growth conditions		Avg repression
		Minimal medium	Minimal medium + DL-methionine (2×10^{-3} M)	
				%
4094-B	eth_1^s eth_2^s	0.381	0.125	66
CH82-7A	eth_1^r eth_2^s	0.405	0.141	
CC30-1D	eth_1^s eth_2^r	0.359	0.333	3
CH82-7D	eth_1^r eth_2^r	0.311	0.316	
D6	? eth_2^s		0–0.050	
CC92-8D	eth_1^r eth_2^r		0	

[a] Expressed as nanomoles per minute per milligram of protein.

[b] Strains are grown in the presence of minimal medium complemented with adenine and uracil. Their genotype is given only regarding the ethionine resistance alleles known to be present. Complete genotypes are given in Materials and Methods

TABLE 2. *Homocysteine synthetase activity[a] and repression in different strains of S. cerevisiae[b]*

Strains	Growth conditions		Avg repression
	Minimal medium	Minimal medium DL-methionine (2×10^{-3} M)	
			%
Haploids			
4094-B(eth_1^s eth_2^s)	444	62	
CH82-7A(eth_1^r eth_2^s)	425	74	87
D6(? eth_2^s)	162	0	
CC30-1D(eth_1^s eth_2^r)	416	236	
CH82-7D(eth_1^r eth_2^r)	525	273	31
CC92-8D(eth_1^r eth_2^r)	214	172	
Heterozygous diploids			
CC18-CC53 (eth_2^s/eth_2^r)	343	67	81
Homozygous diploids			
CC17(eth_2^r/eth_2^r)	248	153	42

[a] Expressed as nanomoles per minute per milligram of protein.

[b] See legend of Table 1. The mutant strains D6 and CC92-8D are grown in the minimal medium described, supplemented with *O*-acetyl-DL-homoserine 10^{-3} M. For heterozygous diploids, we have given the mean value obtained for two homologous diploids.

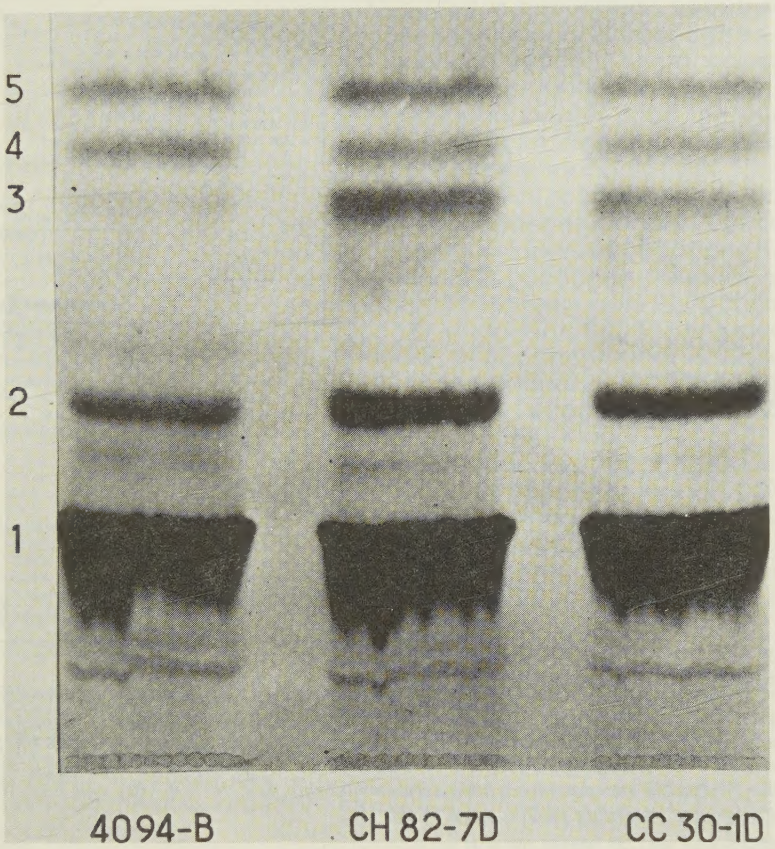


FIG. 1. *Homoserine-O-transacetylase activity in different strains grown in a methione-supplemented medium. Strains used are of the following genotypes: 4094-B: α, ade$_2$, ura$_1$, eth$_1^s$, eth$_2^s$;* CH82-7D: *α, ade$_2$, ura$_1$, eth$_1^r$, eth$_2^r$;* CC30-1D: *α, ade$_2$, ura$_1$, eth$_1^s$, eth$_2^r$. Minimal medium was supplemented with* DL*-methionine 2 × 10^{-3}* M*. Spot identification: 1, ^{14}C-4-*DL*-homoserine; 2, O-acetyl-*DL*-homoserine; 3, compound (ninhydrin-negative) derived from O-acetyl-homoserine; 4 and 5, radioactive impurities present in ^{14}C-4-labeled-*DL*-homoserine. The assay and solvent system have been described (25).*

Garner (37). However, in view of our previous finding of the occurrence of homoserine-*O*-transacetylation in *S. cerevisiae* we favored *O*-acetylhomoserine as a candidate for direct homocysteine synthesis (25). More recently Wiebers and Garner (38) found that acyl derivatives of homoserine are better substrates than homoserine for homocysteine synthesis in *N. crassa*, *Escherichia coli*, and in commercial yeast (38).

We investigated the properties of this enzyme in our strains grown in synthetic or methionine-supplemented media (Table 2 and Fig. 2).

It can be first pointed out that the specific activities found for cells grown in synthetic medium were much higher than activities observed by previous authors for commercial cells (360 nmoles per min per mg of protein at 30 C). This could be caused by the fact that enzymes obtained from commercial cells are often found at their repressed level. This was found to be the case for aspartate kinase, aspartic semialdehyde dehydrogenase, homoserine dehydrogenase, and threonine deaminase (de Robichon-Szulmajster, *unpublished data*).

It can be seen in Fig. 2A that the affinity of the enzyme for *O*-acetyl-L-homoserine (K_m = 7 × 10^{-3} M) is of the same order of magnitude as the one already published (38). However, the enzyme of our wild-type strains exhibited a much higher affinity for methionine (K_i for L-methionine = 1.9 × 10^{-3} M) than that found by Wiebers and Garner (38; Fig. 2B, 2C). In addition, the inhibition caused by methionine seems, in our experiments, competitive with *O*-acetyl-homoserine.

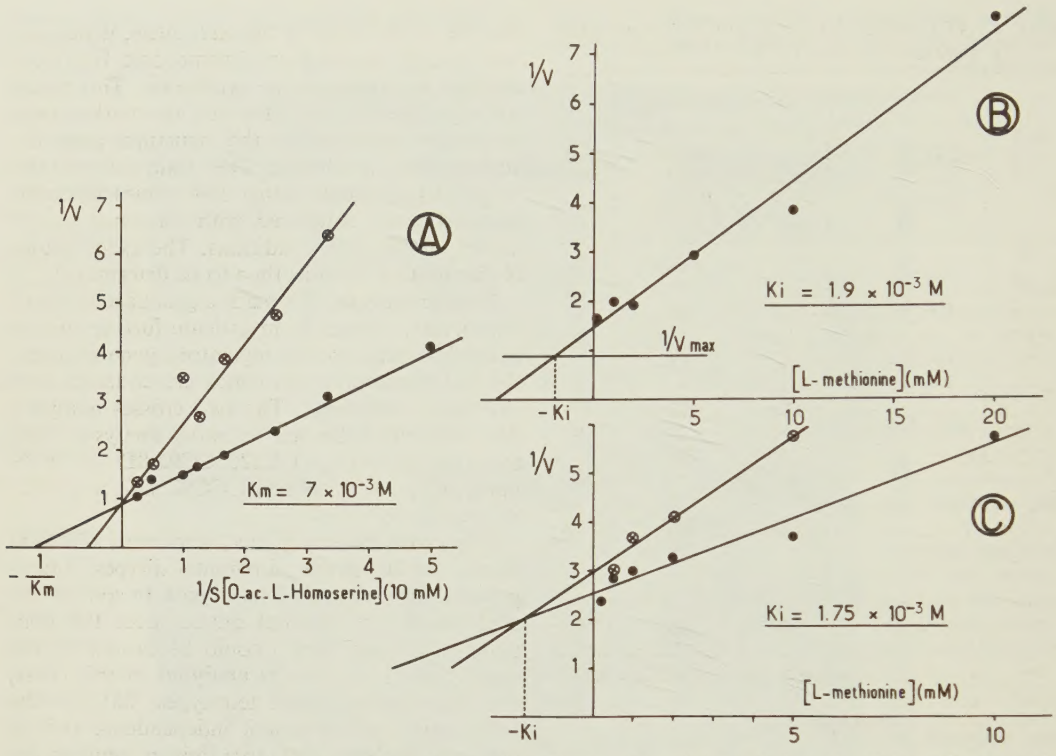


FIG. 2. *Kinetic studies of homocysteine synthetase from S. cerevisiae. (A) Variation of activity with O-acetyl-L-homoserine concentration in the absence (●) or in the presence (⊗) of L-methionine 5 × 10⁻³ M [Lineweaver-Burk transformation (15)]. (B) Variation of activity (1/V) with L-methionine concentration in the presence of O-acetyl-L-homoserine 10⁻² M. Same enzyme preparation was used for A and B. (C) Variation of activity (1/V) with L-methionine concentration in the presence of O-acetyl-homoserine 4 × 10⁻³ M (●) and 8 × 10⁻³ M (⊗). K_i values have been calculated according to Dixon and Webb (4).*

Our results with homoserine-*O*-transacetylase regulation and the role of eth_2^r prompted us to search for homocysteine synthetase repressibility.

Methionine was a very potent repressor of homocysteine synthetase (Table 2) in strains carrying the wild-type allele eth_2^s (87% repression by DL-methionine 2 × 10⁻³ M). As in the case of homoserine-*O*-transacetylase, it appears that the presence of the mutated allele eth_2^r strongly diminished this repression (31%), whereas the state of the eth_1 gene had no effect. In all cases, methionine inhibition has been found to be unmodified.

Homoserine dehydrogenase. It has already been shown that homoserine dehydrogenase synthesis is regulated by methionine (12). In view of our findings on the role of eth_2 gene in the repressibility of homoserine-*O*-transacetylase and homocysteine synthetase, we investigated the repressibility of homoserine dehydrogenase in the two extreme genotypes. Results have shown no difference in this regard (eth_1^s eth_2^s:41 to 45%; eth_1^r eth_2^r:35 to 52% repression by DL-methionine 5 × 10⁻³ M).

ATP sulfurylase. The first step in sulfur assimilation in yeast is repressed by methionine (35). Table 3 shows that repression of ATP-sulfurylase by DL-methionine (2 × 10⁻³ M) was identical in all strains, regardless of the *eth* alleles present.

The specific activities found in these experiments were much higher than the values published by deVito and Dreyfuss (35). Again, strains used and culture conditions are quite different.

As for the other enzymes tested, inhibitory properties of ATP-sulfurylase (here by sodium sulfide) were identical in all cases.

Dominance of the allele eth_2^s on eth_2^r. Earlier genetic and physiological studies, based on ethionine resistance-sensitivity of diploid strains carrying eth_2 alleles in homoallelic or heteroallelic combinations, have shown a complete dominance of eth_2^s (24). Because of the knowledge gained on the regulation of homocysteine synthetase we were able to use repressibility of this enzyme as a criterion of dominance of eth_2 alleles.

The repression of homocysteine synthetase in heteroallelic strains (eth_2^s/eth_2^r) was of the same

TABLE 3. *ATP-sulfurylase activity[a] and repression in different strains of S. cerevisiae[b]*

Strains	Growth conditions		Avg repression
	Minimal medium	Minimal medium + DL-methionine (2×10^{-3} M)	
			%
Haploids			
4094-B(eth_1^s eth_2^s)	148	15	
CH82-7A(eth_1^r eth_2^s)	81	0	93
D6(? eth_2^s)	130	12	
CC30-1D(eth_1^s eth_2^r)	181	12	
CH82-7D(eth_1^r eth_2^r)	246	37	81
CC92-8D(eth_1^r eth_2^r)	230	81	
Heterozygous diploids			
CC18-CC53 (eth_2^s/eth_2^r)	131	5	96
Homozygous diploids			
CC17(eth_2^r/eth_2^r)	168	40	75, 5

[a] Expressed as nanomoles per minute per milligram of protein.
[b] See legend of Table 2.

order of magnitude as that found in haploid eth_2^s strains (Table 2). In addition, in the homoallelic diploid eth_2^r/eth_2^r, the repression was comparable to that found in haploid eth_2^r strains. These data confirm our previous conclusion on the dominance of eth_2^s over eth_2^r.

The results of a control experiment showed that the presence of eth_2^r did not affect the repressibility of ATP-sulfurylase in haploid or diploid strains (Table 3).

Enzymatic study of mutant strains *met*$_8$ and *met*$_{10}$. In a survey of methionine auxotrophs, we estimated the activity of ATP-sulfurylase and homocysteine synthetase (Table 4). It appears that the strain carrying the *met*$_8$ allele, which has been already mapped on chromosome II (19), is deficient in homocysteine synthetase. This result is compatible with the idea that the marker *met*$_8$ represents mutation in the structural gene for homocysteine synthetase. The strain carrying the *met*$_{10}$ allele presents rather low values for both enzymes when compared with reference strains grown in the same conditions. The exact nature of this mutant remains then to be determined.

Genetic analysis. We made a genetic analysis of appropriate crosses to investigate further the relationship between the regulatory gene eth_2 and the two structural genes which are under its control (*met*$_8$ and *met*$_a$). The two crosses analyzed thus far were subjected to tetrad analysis. They are the following: CC125:CC92-8D $\times$ EY9 (*met*$_a$; eth_2^r $\times$ *met*$_8$; eth_2^s) CC92:D6 $\times$ CH82-9C (*met*$_a$; eth_2^s $\times$ eth_2^r).

The cross involving *met*$_a$ and *met*$_8$ (CC125) shows, for 20 tetrads: 6 parental ditypes, 3 nonparental ditypes, and 11 tetratypes. In spite of the slight excess of parental ditypes over the nonparental ditypes, which could be caused by the small number of tetrads analyzed in this cross, the large percentage of tetratypes (55% of the total) is in favor of genetic independence and, in any case, excludes any close linkage between the two genes.

The cross involving *met*$_a$ and eth_2^r (CC92) produced 88 *met*$^-$ and 88 *met*$^+$. Since sensitivity to ethionine is never manifested in the presence of methionine (18), the distribution of the eth_2^r allele could not be tested in the first class. Among the second class, we obtained 44 ethionine-resistant spores. This segregation is typical of two independent characters.

Segregation of the alleles *met*$_8$ and eth_2^r was considered thus far only in the cross described above (CC125). Since this cross involved two independent methionine auxotrophic characters,

TABLE 4. *Enzymatic activities[a] in various methionine auxotrophs[b]*

Strains	Genotypes	Growth conditions (minimal medium + DL-homocysteine, 10^{-3} M)			
		ATP-sulfurylase		Homocysteine synthetase	
		Avg	Extreme values	Avg	Extreme values
EY 9	*met*$_8$ MET$_{10}$	109	82–140	4	0–9
X 1266/1C	MET$_8$ *met*$_{10}$	29	6–42	100	75–171
Reference	MET$_8$ MET$_{10}$	121	62–240	279	109–450

[a] Expressed as nanomoles per minute per milligram of protein.
[b] Complete genotypes are given in Materials and Methods. Since the true parental strains of these two mutants were not in our possession, we have taken as reference activities the average of determinations made on related methionine auxotrophs which are blocked at other steps in the pathway.

only 17 methionine prototrophs were recovered. These yielded 7 ethionine-sensitive and 10 ethionine-resistant spores. Again, this segregation is in favor of independence between the two characters.

DISCUSSION

The results presented in this paper concern two different aspects of methionine biosynthesis in *S. cerevisiae*. One is the direct synthesis of homocysteine, the other is the more general problem of relationship between structural and regulatory genes in a multichromosomal organism like *S. cerevisiae*.

Homocysteine synthetase has already been described in yeast. We found this enzyme at a very high level in our wild-type strains grown in minimal medium. In addition, a completely methionineless mutant, devoid of homocysteine synthetase, was found and characterized. The activity of this enzyme is under strict control by methionine (37, 38). Furthermore, the gene eth_2, which affects repressibility of homoserine-*O*-transacetylase, also affects repressibility of homocysteine synthetase.

Flavin and Slaughter (7) have reported the ability of *Salmonella* cystathionine γ-synthetase to carry out additional reactions, one which is direct synthesis of homocysteine from *O*-succinyl-homoserine and H_2S.

However, recent data from Sorsoli et al. (32) indicate that, although cystathionine is actively transported and the β-cleavage enzyme can be demonstrated in *S. cerevisiae*, the strains studied are unable to use exogenous cystathionine for growth. Giovanelli and Mudd (9) have reported that, in crude extracts of spinach, the conversion of *O*-acetyl-homoserine to *S*-adenosyl-homocysteine is at least 10 times more efficient than its conversion to cystathionine. In addition, the same investigators (10) show that cystathionine fails to decrease the label of homocysteine synthesized from $^{35}SO_4$ and that cystathionine is not labeled in the process. Utilization of cystathionine ^{14}C-labeled in the four-carbon chain has shown that this chain is not incorporated in methionine by *Neurospora* (36).

These results, in addition to (i) the existence of a very active homocysteine synthetase, (ii) the regulatory properties of this enzyme, and (iii) the existence of the mutant EY 9 already described, lead to the obligatory participation of homocysteine synthetase as a step in methionine biosynthesis in *S. cerevisiae* and make it unnecessary to implicate cystathionine as an obligatory intermediate in this pathway.

These considerations led us to favor the following scheme of methionine biosynthesis in *S. cerevisiae* which excludes, thus far, cystathionine as an obligatory intermediate.

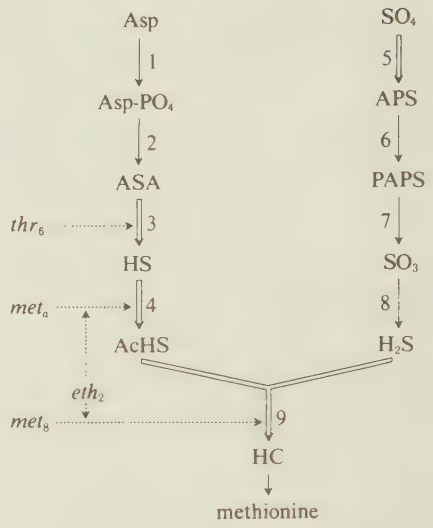


FIG. 3. *Metabolic and regulatory scheme for methionine biosynthesis in S. cerevisiae. Step numbers correspond to the following enzymes: 1, aspartate kinase (L-aspartate 4-phosphotransferase; EC 2.7.2.4). 2, ASA dehydrogenase [L-aspartate-β-semialdehyde: NADP oxidoreductase (phosphorylating); EC 1.2.1.11]. 3, HS-dehydrogenase (L-homoserine: NAD oxidoreductase; EC 1.1.1.3). 4, L-Homoserine-O-transacetylase (Nagai and Flavin, 20). 5, ATP-sulfurylase (ATP: sulfate adenylyl transferase; EC 2.7.7.4). 6, APS-kinase (ATP: adenylsulphate-3'-phosphotransferase; EC 2.7.1.25). 7, PAPS-reductase (Pasternak et al., 21). 8, Sulfite reductase (hydrogen sulphide: NADP oxidoreductase; EC 1.8.1.2). 9, Homocysteine synthetase (Wiebers and Garner, 38). Relevant structural genes are connected to reactions by broken arrows. Compounds are abbreviated as in the scheme given in the introduction. Large empty arrows indicate enzymatic steps repressed by methionine.*

Although a few examples of clustered structural genes have been reported (5, 6, 14, 34) in *S. cerevisiae*, it is commonly found that genes controlling sequential steps in a metabolic pathway are located on different chromosomes (19). This is the case for the threonine-methionine biosynthetic pathway studied in this laboratory (23, 28).

Data obtained by de Vito and Dreyfuss (35) and from this laboratory (12, 25) have shown that the synthesis of four enzymes involved in methionine biosynthesis in *S. cerevisiae* responds to exogenous methionine (indicated by large arrow on Fig. 3). Results presented in this paper show that the regulation of two of these enzymes (homoserine-*O*-transacetylase, step 4, and homocysteine synthetase, step 9) is under the control of the gene eth_2, whereas regulation of the two other

enzymes (homoserine dehydrogenase, step 3, and ATP-sulfurylase, step 5) is not.

Previous genetic analysis has shown that ethionine resistance conferred in haploid strains by the mutant allele eth_2^r is recessive in the heterozygous diploid, eth_2^s/eth_2^r. Present data confirm the dominance of the eth_2^s allele when characterized by assay of homocysteine synthetase repressibility on various diploid strains. In addition, it is shown that eth_2 segregates independently of the gene met_a and of the gene met_8, and that met_a and met_8 are unlinked as well.

These findings suggest that the eth_2 gene may be involved in the synthesis of a pleiotropic methionine repressor on two unlinked structural genes met_a and met_8. The two other enzymes, whose synthesis is repressible by exogenous methionine but is nevertheless unaffected by eth_2, must then remain under the control of another (or other) methionine repressor(s).

Different types of repressor formed responding to the presence of a single amino acid can be postulated. They could differ either by the nature of the apo-repressor or by the exact nature of the corepressor: free amino acid, derivatives, and activated forms. In the case of methionine, two activated forms can be considered: the methionyl-transfer ribonucleic acid and the sulfonium derivative, *S*-adenosyl-methionine (SAM). The latter is accumulated in the presence of exogenous methionine (31), whereas the free methionine pool remains, in contrast, remarkably constant under these conditions (H. Cherest, *unpublished data*). It can be recalled that homoserine-*O*-transacetylase is inhibited by SAM and is insensitive to free methionine. We suggested that, by analogy, the corepressor of the gene met_a might also be SAM (25). Homocysteine synthetase, on the other hand, is very sensitive to inhibition by free methionine. The quantitative difference in the derepression effect on these two enzymes, in response to eth_2^r, could be due to a slight difference between the two operator-like structures of the two structural genes or to a slight difference in the nature of the corepressors involved.

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